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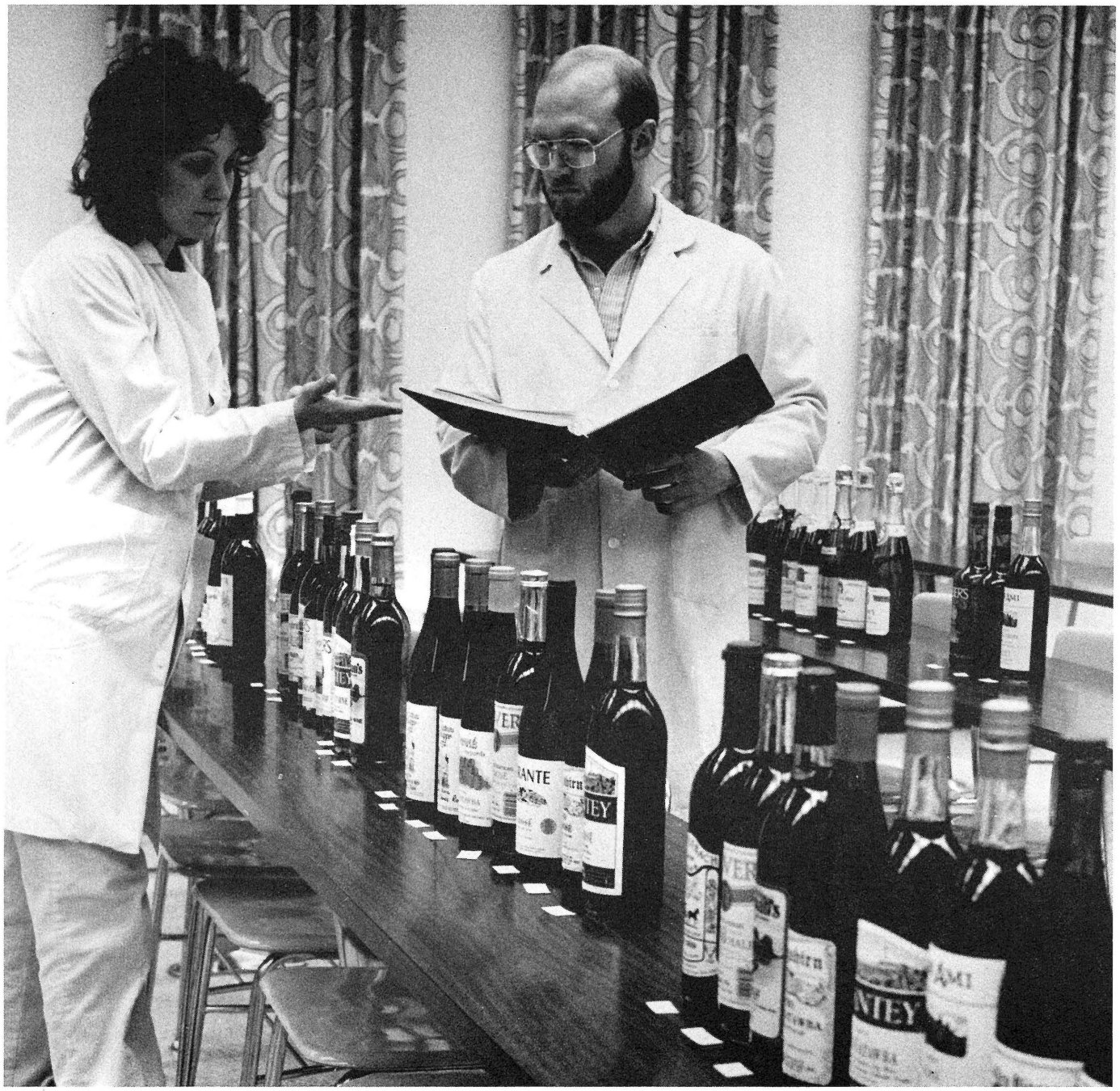

The Ohio State University
Ohio Agricultural Research and Development Center
Wooster, Ohio

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OARDC

Kirklyn M. Kerr, Director

On the cover: This display of Ohio commercial wines is being prepared for evaluation at the annual Ohio Wine Conference. This event is usually held at the OSU/OARDC in Wooster with more than 190 entries.

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Higher Alcohol Formation In Wines as Related to Nitrogen Fertilization and Alar Application to Concord Grapevines

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Introduction

During alcoholic fermentation, small amounts of higher alcohols are produced by yeasts. These alcohols have greater molecular weights than ethanol and include alcohols such as n-propyl, isobutyl, act-amyl, and isoamyl. They are produced throughout the course of alcoholic fermentation with greater amounts formed at the end of the fermentation (Castor and Guymon, 1952). The formation of higher alcohols in wines is associated, in part, with certain amino acids, mainly leucine, isoleucine, and valine (Guymon, 1972). Castor and Guymon (1952) reported that these amino acids are utilized early in the yeast fermentation, and when exhausted, another pathway gives rise to higher alcohol formation.

With regard to their influence on wine quality, small amounts of higher alcohols contribute to wine complexity, while higher levels tend to lower wine quality. For eastern table wines, levels of higher alcohols over 300 mg/L tended to decrease the quality of the wines (Liu *et al.*, 1987). Also, Wagener and Wagener (1968) reported that large amounts are detrimental to wine aroma, especially where the total ester is low.

Nitrogen is an essential element of plants and is routinely applied to vineyards to improve and maintain grapevine vigor. However, in some instances where a vineyard has not achieved its full capability, the application of Alar has been found to improve productivity. This growth regulator has been shown to increase yields, about 1 ton per acre is quite typical for certain grape varieties.

Since nitrogen fertilization and Alar application may influence the amino acid composition of grapes, the purpose of this study was to investigate the effects of two cultural practices on higher alcohol formation.

Materials and Methods

Vineyard: The Concord grapes used were from an experimental vineyard located at OARDC, Wooster, Ohio. The grapes were harvested from a factorial design experiment of nitrogen and Alar. For the nitrogen applications, three levels were used: 50, 100 and 150 lb/acre, which were applied in early spring as ammonia nitrate. Grapes receiving 150 lb/acre of nitrogen were treated with 0, 750, and 1500 mg/L Alar at first bloom. Grapevines were trained to the Geneva Double Curtain and were spaced 2.4 m between vines and 3.1 m between rows.

Vinification: From each of these grape plots, approximately 180 lb of grapes were harvested at peak maturity. These grapes were divided into three lots and each lot was destemmed, crushed, and pressed in a Willmes press for 25 min. at 4 bars. Juice (8 L) from each lot was treated with 75 mg/L sulfur dioxide and ameliorated with 71° Brix syrup (H.F.) to 25° Brix. After 12 h, each juice was inoculated with Montrachet #522 yeast and fermented at 21°C. When the fermentation was completed, the wines were racked and transferred to 2°C storage for three weeks. After cold stabilization, the wines were sulfited, racked, bottled, and analyzed.

Chemical Analysis: Titratable acidity, pH, °Brix, and total nitrogen were analyzed as described by Amerine and Ough (1980). For the analysis of free amino acids, juices were prepared, and the acids were determined with amino acid analyzer. Higher alcohols were analyzed by the gas chromatographic procedure of Martin *et al.* (1981).

Results and Discussion

Table 1 gives the composition of the musts from the five treatments. The mean values for pH and titratable acidity did not differ among the treatments. However, both °Brix and total nitrogen levels of the musts were influenced by the cultural treatments. For the nitrogen treatments, the results indicated that the °Brix values increased with the rise of nitrogen fertilization. This response was expected, for these vines were overcropped, stressed, and the addition of nitrogen to the vines caused an increase in leaf area and photosyn-

Table 1. Effect of nitrogen fertilization and Alar application on juice composition of Concord grapes.

Constituent[1]	Nitrogen (lb/acre) 50	100	150	Nitrogen (150 lb/acre) plus Alar (mg/L) 750	1500
°Brix	14.1	14.7	15.1	14.3	13.5
pH	3.30	3.34	3.33	3.33	3.33
T.A.[2]	0.69	0.65	0.67	0.67	0.67
Nitrogen (mg/L)	700	900	900.	1000	700
Amino Acids					
Alanine (mg/L)	290	344	304	340	259
Arginine (mg/L)	148	192	213	221	184
Glutamine (mg/L)	49	49	50	43	40
Threonine (mg/L)	24	35	29	21	57
Glutamic acid (mg/L)	298	296	321	580	678
Valine (mg/L)	8	11	14	9	8
Leucine (mg/L)	5	6	5	4	4
Proline (mg/L)	22	32	29	47	28
Isoleucine (mg/L)	3	3	3	3	3
Aspartic acid (mg/L)	17	15	16	18	17
Serine (mg/L)	17	16	25	21	21
Total A.A. (mg/L)	881	999	1009	1307	1298

[1]Each value is the mean of three replicates.
[2]Titratable acidity as g tartaric acid per 100 ml.

thesis which resulted in an increase in °Brix, sugar content. Effects of nitrogen fertilization on vines with normal crop load would be expected to show no response or depress fruit maturity. The reduction in sugar content with Alar applications was probably due to the increase in berry set and crop load.

The increase in amounts of nitrogen fertilizer did result in parallel increase of nitrogen in the musts. The total free amino acids also increased in the musts as the level of nitrogen fertilization was increased. The major amino acids, alanine, arginine, and glutamic acid, tended to increase, while the other acids remained fairly constant with increasing nitrogen applications. The most striking observation in this study was the large increase in alanine, arginine, and proline at the lowest level of Alar application (750 mg/L). At the highest Alar application (1500 mg/L), the concentrations of glutamic acid and threonine were increased substan-tially in the must.

Formation of higher alcohols in the wines was found to be related to the nitrogen fertilization of the vines (Table 2). The concentrations of n-propyl and isobutyl alcohols were highest in wines made from grapes which received the highest nitrogen treatment. For act-amyl and isoamyl alcohols, their amounts in wines showed a decrease with increasing nitrogen fertilization. In general, these findings are in agreement with the results of Ough and Bell (1980). However, their study indicated a very slight decrease in isobutyl alcohol with nitrogen fertilization.

Wines from grapes that were treated with Alar at 150 lb N/acre were found to contain less amounts of n-propyl and isobutyl alcohols than those made from grapes receiving no Alar (Table 2). For act-amyl and isoamyl alcohols, the results indicated that both alcohols increased with higher levels of Alar applications. Although previous investigations have reported that larger amounts are derived from leucine, isoleucine, and valine (Kliewer *et al.*, 1966; Ough and Bell, 1980), these amino acids were found in very small amounts and were fairly constant in berries treated with Alar. The Alar treatments seemed to cause changes in amino acid composition of musts (Table 1), but no trends were noted between individual acids and higher alcohol formation.

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Table 2. Effect of nitrogen fertilization and Alar application on higher alcohol formation in Concord wines.

Alcohol[1]	Nitrogen (lb/acre)			Nitrogen (150 lb/acre) plus Alar (mg/L)	
mg/L)	50	100	150	750	1500
N-Propyl	21.8	24.1	24.1	23.5	22.4
Isobutyl	34.2	37.9	40.4	39.1	39.4
Act-Amyl	20.7	19.7	19.1	22.2	22.0
Isoamyl	304.4	283.3	278.6	284.7	299.8

[1]Each value is the mean of three replicates.

Influence of Vineyard Location, Maturation, and Season on the Chemical Composition of Ohio Concord Grapes

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Introduction

The Concord grape is the best known and most important variety grown in Ohio. It is widely planted throughout Ohio and is mainly used for various grape products, especially processed juice and wine. Although considerable information has been devoted to the chemical composition of grapes, little is known about those factors which effect the chemical components of Concord grapes in Ohio. In general, the chemical composition is the most important criterion used in determining the quality of this fruit for processing. Like most fruits, chemical changes in grape composition can be influenced by climate, maturation, and season. Information about these factors and other practices and causes is essential in evaluating fresh fruit quality processing grape products.

The present study was undertaken to determine the effect of maturity, season, and vineyard location on the composition of Ohio Concord grapes and juices. Berries from five vineyard sites were analyzed at three stages of maturity for two seasons, 1982 and 1983.

Materials and Methods

Berry samples of Concord grapes were collected in 1982 and 1983 from five different locations in Ohio. At each location, 10 vines were selected for collecting samples at three maturity levels (°Brix) which represented early-, mid-, and late-stages of maturity.

For each maturity, two berries were collected from each of 20 clusters which were located in the middle of the upper portion of the vine. The berries from 10 vines were pooled, and the procedure was repeated twice.

Juice Preparation: One hundred berries were weighed and then passed through a screw press (Squeezo strainer) to obtain free-run juice. Approximately 50 mL free-run juice was centrifuged on an IEC-140 rotor at 900 x g for 10 min to remove the gross particles.

For the heat-extracted juice, approximately 200 g of berries were macerated in a Waring blender at low speed for 1.5 min. The mixture was placed in a 250 mL beaker, covered with a watch glass, and heated in a water bath at 85°C for one hour. The heated macerate was cooled to 40°C and drained through cheesecloth. The heat-extracted juice was then centrifuged as described for the free-run juice.

All analyses were performed on freshly extracted juices, free-run and heat extracted, except for the determination of organic acids and minerals. For these constituents, frozen samples were prepared immediately after extraction. The juice samples were diluted with an equal volume of deionized water and stored in glass containers at -17°C. At the time of analysis, the frozen samples were thawed and heated in a water bath at 85°C for one hour and cooled to room temperature.

Chemical Analysis: Titratable acidity, pH, °Brix and tartaric acid (metavanadate procedure) were analyzed as described by Amerine and Ough (1980). L-malic acid was determined by the enzymatic procedure of Mayer and Busch (1963). Analyses of inorganic elements were performed by a direct reading emission spectrograph. Color was determined by diluting 1 mL of juice to 10 mL with pH 1.0 KCl-HCl buffer and further diluted 1:20 in order to measure to optical density (OD). After filtering through an 0.45μ membrane filter, samples were read at 420 nm and 520 nm in a Perkin-Elmer Lambda spectrophotometer.

Results and Discussion

The data for the free-run juice from Concord grapes is shown in Table 1. In general, berry weights increased with fruit maturation and indicated that berry size was greatest in 1983. It is well known that the total amount of water increases during ripening and certain fluctuations occur among seasons, due to the amount of rainfall (Amerine, 1956). The pH and °Brix increased, while titratable acidity decreased during fruit development. The highest °Brix

levels were observed in 1983, which yielded the smallest berries. The decrease in titratable acidity during maturation is reflected by a reduction in total tartrates and malates. Several investigations have shown that both organic acids decrease during fruit ripening with malic acid decreasing the most. At peak maturity, late harvest, tartaric acid was the predominant acid and finding is in agreement with previous findings (Amerine and Winkler, 1942; Kliewer *et al.*, 1967). Since the 1983 season was the best year for sugar (°Brix) accumulation, it was predictable that the lowest level of titratable acidity would occur in this season. Although past investigations have shown that potassium increases with fruit ripening (Amerine, 1956; Puissant, 1960), results of this study found no relationship between potassium and grape maturation. Also, no differences were observed in potassium levels of grapes in both seasons. Calcium and magnesium levels were found to decrease with fruit development. Puissant (1960) also reported that calcium and magnesium contents decreased significantly as the season progressed.

The composition of heat-extracted juices differed from the free-run juices. The heat treatment increased the level of all constituents which resulted in higher °Brix values (Table 2). Since °Brix is a measure of soluble solids, higher °Brix readings were due to the increase in nonsugar components, mainly organic acids, minerals, and pigments. The heat treatment caused a substantial increase in tartrates and moderate rise in malates. Thus, Concord grapes contain more tartrate than malate in the skins. Also, inorganic components were found to be highest in heat-extracted juices. The increase in inorganic cations may explain the higher pH values in the heat-extracted juices than the free-run samples. Color readings, OD 420 nm and 520 nm, indicated that color intensity paralleled fruit development. The highest color values were obtained at peak maturity. Also, the heat-extracted juices from northern Ohio were found to have more color. Winkler and Amerine (1938) reported that grapes from warm regions, other factors being equal, tended to have less color. Since color is one of the most important quality attributes of wine and juice, processors should consider grapes from northern Ohio to increase their product quality. In addition, northern Ohio grapes tended to have the highest sugar concentration, °Brix, which processors consider essential in making high quality juice and wine products.

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Table 1. Chemical composition of free-run juice from Concord grapes from five vineyard locations at three maturity levels for two seasons, 1982 and 1983.

Ohio Vineyard location	Harvest date	Season	Wt/100 berries g	°Brix	TA[1]	pH	K mg/L	Ca mg/L	Mg mg/L	Tartrate g/ 100 mL	Malate g/ 100 ml
Morrow	8/31	1982	333	11.8	0.86	2.99	1243	36	46	0.61	0.47
(South)	9/7		344	13.9	0.77	3.18	1397	37	49	0.51	0.38
	9/15		350	15.7	0.64	3.28	1259	47	50	0.40	0.26
	9/1	1983	308	12.6	1.00	3.12	1487	46	55	0.64	0.49
	9/12		309	14.9	0.74	3.28	1543	48	56	0.51	0.22
	9/21		324	16.4	0.56	3.43	1552	43	55	0.41	0.15
Columbus	8/31	1982	409	12.4	1.03	2.97	1347	35	45	0.54	0.61
(Central)	9/7		404	14.5	0.93	3.17	1798	41	56	0.47	0.53
	9/15		411	15.2	0.92	3.24	2235	45	63	0.57	0.45
	9/6	1983	329	13.7	0.90	3.17	1667	48	56	0.59	0.46
	9/14		334	15.1	0.65	3.28	1553	44	51	0.50	0.28
	9/21		333	16.3	0.63	3.37	1666	51	60	0.50	0.28
Wooster	8/30	1982	309	11.7	1.78	2.92	1954	42	64	0.65	1.00
(North	9/9		354	13.4	1.08	3.11	1944	33	58	0.42	0.68
Central)	9/28		367	15.6	0.66	3.30	1316	37	52	0.30	0.35
	9/5	1983	243	14.6	0.97	3.02	1408	66	64	0.78	0.41
	9/14		261	17.0	0.71	3.13	1388	58	62	0.55	0.24
	9/21		275	18.1	0.57	3.00	1246	48	53	0.53	0.18
Sandusky	9/1	1982	313	14.2	1.16	2.99	1577	43	62	0.67	0.63
(North)	9/13		279	15.1	0.66	3.19	977	37	47	0.46	0.32
	9/23		304	15.9	0.53	3.21	1004	32	47	0.25	0.26
	9/7	1983	222	14.1	0.78	3.19	1410	47	62	0.68	0.34
	9/15		278	15.7	0.56	3.25	1520	52	69	0.60	0.24
	9/22		264	16.1	0.34	3.38	1010	39	41	0.28	0.14
Madison	9/9	1982	293	14.0	1.20	2.80	1225	43	58	0.53	0.56
(North)	9/23		328	15.0	0.64	3.06	987	37	47	0.24	0.27
	10/5		297	17.4	0.71	3.01	1096	35	55	0.39	0.26
	9/8	1983	250	12.9	1.35	2.91	1442	49	65	0.83	0.65
	9/19		266	15.5	0.89	3.06	1385	42	60	0.64	0.40
	9/27		291	16.6	0.62	3.23	1108	36	46	0.35	0.30

[1]Titratable acidity as g tartaric acid per liter.

Table 2. Chemical composition of heat-extracted juice from Concord grapes collected from five vineyard locations at three maturity levels for two seasons, 1982 and 1983.

Ohio Vineyard location	Harvest date	Season	°Brix	TA[1]	pH	K mg/L	Ca mg/L	Mg mg/L	Tartrate g/ 100 mL	Malate g/ 100 mL	OD 420 nm	OD 520 nm
Morrow	8/31	1982	13.7	1.29	3.16	2628	80	102	1.09	0.69	.047	.111
(South)	9/7		14.6	1.08	3.30	2313	70	101	0.81	0.46	.050	.106
	9/15		17.4	1.02	3.32	2603	73	98	0.84	0.36	.084	.216
	9/1	1983	13.0	1.31	3.14	2523	95	89	1.06	0.59	.007	.014
	9/12		15.8	0.98	3.33	2861	92	106	1.03	0.32	.035	.069
	9/21		16.9	0.80	3.44	2855	78	97	0.91	0.25	.035	.110
Columbus	8/31	1982	14.3	1.51	3.10	2697	84	99	1.15	0.78	.048	.130
(Central)	9/7		15.8	1.27	3.22	2432	76	96	0.91	0.65	.072	.206
	9/15		17.2	1.17	3.29	2665	83	108	0.90	0.54	.102	.240
	9/6	1983	14.2	1.19	3.27	3032	100	110	0.97	0.57	.037	.063
	9/14		15.6	0.97	3.36	2975	87	108	0.88	0.40	.023	.064
	9/21		16.6	0.87	3.40	3038	85	105	0.88	0.34	.034	.105
Wooster	8/30	1982	12.9	1.80	3.02	2191	93	110	1.19	1.11	.069	.107
(North	9/9		14.4	1.45	3.20	2307	79	113	0.86	0.78	.069	.171
Central)	9/28		17.0	1.19	3.42	1921	64	104	0.80	0.53	.114	.282
	9/6	1983	15.6	1.26	3.10	2467	126	132	1.18	0.44	.074	.140
	9/14		17.2	1.04	3.17	2548	108	126	1.08	0.33	.053	.155
	9/21		18.5	0.95	3.08	2604	93	114	0.97	0.27	.087	.193
Sandusky	9/1	1982	15.6	1.46	3.10	2652	96	138	0.87	0.67	.056	.179
(North)	9/13		18.0	1.30	3.29	2458	82	128	0.87	0.44	.143	.361
	9/23		18.4	1.24	3.34	1906	68	99	0.91	0.40	.105	.310
	9/7	1983	14.9	1.07	3.27	2778	95	123	0.94	0.43	.008	.007
	9/15		16.1	0.90	3.32	2781	81	118	0.81	0.35	.038	.160
	9/22		17.4	0.91	3.38	2828	76	112	0.70	0.24	.052	.157
Madison	9/9	1982	14.7	1.52	2.88	2010	82	103	1.05	0.69	.102	.200
(North)	9/23		16.7	1.13	3.04	2603	74	128	1.06	0.43	.124	.366
	10/5		18.8	1.10	3.04	2038	74	115	1.00	0.32	.170	.542
	9/8	1983	13.3	1.58	3.01	2250	100	120	1.03	0.76	.018	.046
	9/19		16.3	1.16	3.13	2518	82	119	1.02	0.49	.041	.146
	9/27		17.8	1.03	3.24	2581	70	107	0.92	0.44	.061	.214

[1]Titratable acidity as % tartaric acid per liter.

The Influence of Juice Clarification on the Quality of White Table Wines

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Introduction

Freshly pressed juice usually contains a relatively high amount of insoluble solids, which consists of skin and seed particles, and various pulpy substances. Studies of juice clarification on wine quality have indicated that wines produced from clarified juice were higher in wine quality (Singleton *et al.*, 1975; Van-Wyk, 1978; Williams *et al.*, 1978). Singleton *et al.* (1975) reported that wines from clarified juice as being fresh, clean, delicate, and fruity. In addition, this study indicated that these wines lacked off-odors, particularly hydrogen sulfide. Crowell and Guymon (1963) and Groat and Ough (1978) reported that an increase in juice solids cause a higher formation of higher alcohols. Wagener and Wagener (1968) found that high levels of fusel oil in white wines were detrimental to wine quality.

In order to obtain clarified juices prior to alcoholic fermentation, several methods may be used to remove the insoluble solids. These methods include settling, filtration, or centrifugation. The most common practice, especially among small wineries, is the settling method. The purpose of this study was to investigate the effect of this clarification practice on the composition and quality of French hybrid white table wines. There is a lack of information demonstrating the effect of clarification on the wine quality of eastern varieties, particularly French hybrids.

Materials and Methods

Vinification: In 1983, grapes from the varieties Seyval and Vidal blanc were harvested from a commercial vineyard in northern Ohio. The grapes were destemmed, crushed, and treated with 75 mg/L of sulfur dioxide. After a light pressing, the juice from each variety was ameliorated with sucrose to 20° Brix and divided into two lots for the following treatments: 1) control the unclarified juice was racked into a five-gallon carboy and triplicated; and 2) naturally settled: the clarified juice was settled naturally at 16.7°C for 12 hours and then racked into a five-gallon carboy and triplicated. All carboys were equipped with water seals.

For each juice, a 10 mL sample was centrifuged at 900 g for 10 minutes, and the insoluble solids content was determined as percent by volume. All lots were fermented at 18.0°C with Epernay #2 yeast. At dryness, all wines were racked, treated with sulfur dioxide (20 mg/L), and cold-stabilized prior to chemical and sensory analyses.

Analyses: Titratable acidity, pH, °Brix, alcohol, volatile acidity, color, and tartaric acid were analyzed as described by Amerine and Ough (1980). L-malic acid was determined by the enzymatic procedure of Mayer and Busch (1963). A gas chromatographic procedure of Martin et al. (1981) was used to determine the higher alcohols.

The wines were evaluated by a taste panel with a paired comparison test for aroma and taste preference. The taste panel consisted of eight judges. For each variety, there was a total of three tasting sessions. At each session, each judge was asked to express their preference between two vines (unsettled and settled) in each pair. A total of 24 trials were conducted for each pair of wines.

Results and Discussion

The °Brix, pH, titratable acidity and insoluble solids are shown in Table 1. No differences were found between the juices of the two clarification treatments in their °Brix, pH, and titratable acidity levels. However, there were differences in solids contents between the unsettled and settled juices. The settling treatment was found to reduce the insoluble solids to less than 0.5 percent (v/v). The fermentation rates were slower for those wines which were made from settled juices (data not shown in Table 1). Studies by Groat and Ough (1978) indicated that slower yeast fermentations were found in musts with insoluble solids below 0.5 percent (v/v).

For the wines, the results of the chemical analyses indicated that the titratable acidities were slightly higher for wines made from unsettled juices than those produced from settled juices (Table 2). These results also indicated that settling juice prior to

alcoholic fermentation increased the pH of the wines. This relationship is in contrast to most wines which are usually lower in acidity and higher in pH than their corresponding juices. However, Boulton (1980) reported that pH can decrease with lower acidity after fermentation. The alcohol, tartrate, and malate contents were similar between the wines made from unsettled and settled juices. Volatile acidities were highest for wines vinified from settled juices. This increase was expected, for more acetic acid is usually formed with prolonged wine fermentations. The wines from the unsettled juices were slightly higher in color than those from settled juices.

As expected, juice clarification reduced the level of higher alcohols in the wines (Table 2). Groat and Ough (1978) reported that juices containing less suspended solids tended to be lower in higher alcohols. All of the individual alcohols were greatly reduced in the wines by settling except propanol which showed a modest decrease.

A paired comparison test was used to evaluate the wines for aroma and taste. The results indicated that the judges preferred the wines from the settled juices both aroma and taste (Table 3). The panelists described the wines as being more fruity and clean tasting.

Table 1. Juice composition of Vidal blanc and Seyval grapes.

Variety	Clarification treatment	pH	TA[1]	°Brix	Insoluble solids %
Vidal blanc	Unsettled	3.10	0.98	17.3	3.0
	Settled	3.10	0.96	17.3	<0.5
Seyval	Unsettled	3.17	0.74	18.6	2.4
	Settled	3.19	0.74	18.6	<0.5

[1]Titratable acidity expressed as tartaric acid equivalent, grams per 100 mL.

Table 2. Composition of Vidal blanc and Seyval wines.

Constituent	Vidal blanc		Seyval	
	unsettled	settled	unsettled	settled
pH	2.86	2.90	3.08	3.11
TA[1]	0.83	0.79	0.68	0.66
VA[2]	0.033	0.039	0.027	0.047
Alcohol (% v/v)	11.4	11.4	11.8	11.8
Color[3]	0.087	0.082	0.114	0.093
Tartrate (g/100 mL)	0.33	0.30	0.31	0.30
Malate (g/100 mL)	0.33	0.35	0.23	0.23
Higher Alcohols				
N-propyl (mg/L)	22	16	18	15
Isobutyl (mg/L)	41	19	57	28
Act-Amyl (mg/L)	30	19	45	23
Isoamyl (mg/L)	146	105	220	106

[1]Titratable acidity as g tartaric acid per 100 mL.
[2]Volatile acidity as g acetic acid per 100 mL.
[3]Absorbance at 420 nm.

Table 3. Paired comparison test for wines made from unsettled and settled juices.

Variety	Number of samples preferred by taste panelists		
	Paired sample	Aroma	Taste
Vidal blanc	Settled vs. unsettled	18[1] vs. 6	20[2] vs. 4
Seyval	Settled vs. unsettled	23[3] vs. 1	20[2] vs. 4

[1,2,3]significant at the 0.05, 0.01, and 0.001 levels, respectively.

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Pectin Changes During Blueberry Fruit Development

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Introduction

Blueberry production has increased by 90 million tons over the last ten years and many growers are considering blueberries as an alternative crop (Adams, 1987). Work has been done on some aspects of fruit quality including color and flavor (Sapers *et al.*, 1984), whereas, little research has focused on changes in blueberry texture during their development, maturity and senescence.

Alteration of the cell wall and middle lamella of fruits and vegetables is responsible for textural changes during development, senescence, postharvest storage and processing (Eskin, 1979; Huber, 1983). Pectic substances, hemicellulose and cellulose are the major cell wall polysaccharides and they frequently undergo depolymerization in maturing fruit. Depolymerization of cell wall polymers contributes greatly to tissue softening (Eskin, 1979; Huber, 1983). Pectin is the predominant component of the middle lamella which is depolymerized and solubilized during the ripening of many fruits.

As a general observation, unripe fruit contain small amounts of soluble pectin but this increases dramatically with ripening (Huber, 1983). Nevertheless, soluble pectin, with free carboxyl groups, can be rendered insoluble by crosslinking to adjacent polymers with divalent cations such as calcium or magnesium (Eskin, 1979). The objective of this study was to measure total pectin, water-soluble pectin, chelator-soluble pectin and insoluble pectin in developing blueberries.

Materials and Methods

Fruit Sampling and Storage: Random samples of developing, maturing, and senescing "Bluetta" blueberries were taken from six bushes on five occasions over a period from May 29 to June 26, 1987. At each picking the berries were immediately immersed in liquid nitrogen and stored at −23°C prior to pectin analysis. The date of harvest was recorded as days after full bloom.

Alcohol Insoluble Solids Preparation: Alcohol insoluble solids (AIS) were prepared by a method adapted from Woodward (1972). An acidic methanol extraction removed anthocyanins and the remaining solids were refluxed with 80 percent ethanol. The solids were then washed with acetone before a final filtration. The samples were then dried *in vacuo* at 25° overnight, weighed and stored in a desiccator at room temperature.

Pectin Analysis: Total pectin determinations were made according to the method of Ahmed and Labavitch (1977). Fractionated pectic substances were obtained as described by Hudson and Buescher (1985). Aqueous extraction removed water-soluble pectin (WSP), 0.5 percent sodium hexametaphosphate, extracted chelator-soluble pectin (CSP) and 0.05 percent sodium hydroxide, solubilized dilute alkali-soluble pectin (DASP). Total and fractionated pectin was measured as total uronic acid according to the method of Blumenkrantz and Asboe-Hansen (1973), with the modifications of Kinter and Van Buren (1982). Each determination was made in triplicate and was expressed as a percentage of AIS and on a fresh weight basis.

Results and Discussion

Changes in the percent contribution of total cell wall material to fruit fresh weight, as indicated by AIS, are shown in Figure 1. There was a decline in AIS in fruits up to 43 days after full bloom, which was particularly rapid between day 36 and day 43. Loss of AIS coincided with field observations of fruit softening. After 44 days AIS levels stabilized and on reaching maturity at 48 days, there was little change in cell wall material, even if the fruit remained on the plant nine days after the maturity date. Decrease in AIS is probably due to an increase in water uptake and subsequent increase in fruit size. Total pectin varied as a component of AIS material (Figure 2). Between day 30 and 36 there was a reduction in the contribution of total pectin to the cell walls which was followed by a rapid increase in the amount of pectin in the AIS until day 43. Pectin levels declined steadily after day 44, shortly before the harvest date.

Figure 1. Changes in alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. LSD=0.40.

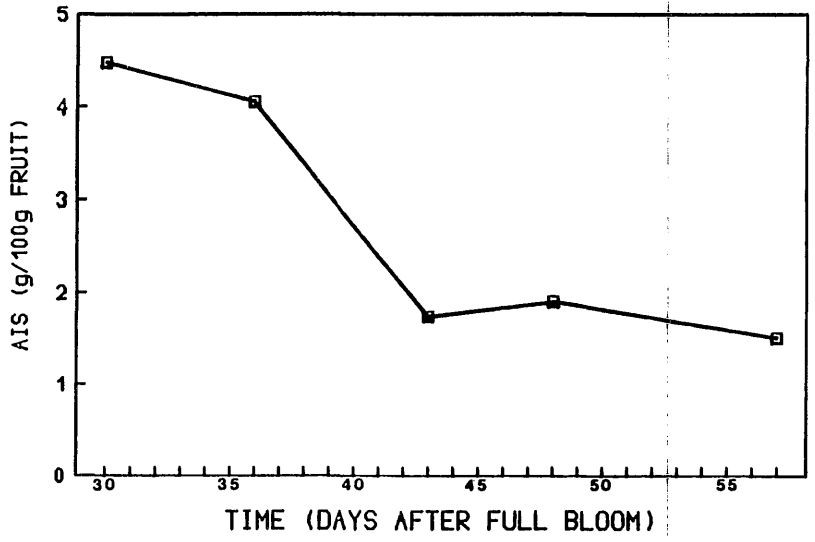
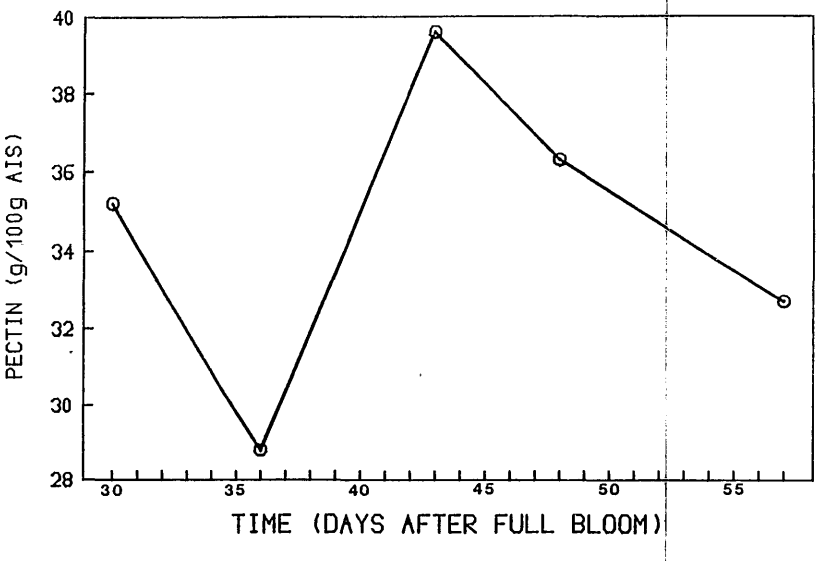


Figure 2. Changes in the total pectin of alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. LSD=0.81.



When pectin content was expressed on a fruit fresh weight basis (Figure 3), it declined steadily until day 43, with little change at maturity at day 48. This may reflect the greater increase in water content, relative to pectin, during growth.

Changes in the levels of fractionated pectic substances during fruit development are shown in Figure 4. The initial rapid increase in DASP corresponded to a large reduction in WSP. After day 36, DASP concentrations fell until day 43, while WSP steadily increased throughout the harvest dates. CSP initially rose with WSP but remained constant after 43 days. Similar trends were observed when the data were expressed on a fresh weight basis (Figure 5). The sum of the fractions only accounted for part of the reported total pectin. This is due to "non-extractable pectin" which has been described as insoluble, highly methylated pectin that is difficult to quantify by differential solubilization techniques (Hudson and Buescher, 1984). However, it is included in total pectin analysis by acid hydrolysis (Ahmed and Labavitch, 1977). The non-extractable pectin constituted 36 percent of the total pectin on days 30 and 43, declined to 23 percent on day 47, and was negligible on day 57. The very small reading of 4 percent non-extractable pectin on day 36 may be an artifact of the experiment.

The data suggest a possible mechanism for pectin metabolism in ripening blueberries. The protopectin, as represented by the DASP fraction, accumulated up to day 36, after which solubilization was initiated resulting in increased amounts of WSP. In achieving this conversion at least two enzymes may be involved, pectinmethylesterase (PME) and

polygalacturonase (PG). Demethylation by PME would result in greater numbers of carboxyl groups which may facilitate PG activity and binding of cations. Polygalacturonase preferentially degrades de-esterified pectin substances, thus PME may prepare the substrate for PG attack (Huber, 1983). Woodruff *et al.* (1960) observed PME activity to increase in maturing blueberries. Rising levels of CSP suggest increasing demethylation, probably as a result of PME activity, and subsequent binding of divalent cations. Furthermore, the increase in the gradient of the WSP curve (Figure 4), as CSP levels stabilized, suggests that the availability of divalent cations may be a limiting factor in CSP formation. The CSP fraction presumably consists of calcium pectate (Hudson and Buescher, 1985). This is supported by a recent finding that the majority of cell wall bound calcium was in the CSP fraction (Howard, 1987). It could be that CSP is formed during AIS preparation when the tonoplast is ruptured. This would allow WSP to bind calcium ions from the cell vacuole.

In conclusion, this study suggests that there is reduction in the pectin content of developing blueberry fruit, probably due to pectin solubilization.

Figure 3. Changes in the total pectin of "Bluetta" blueberries expressed on a fruit fresh weight basis. LSD=0.14.

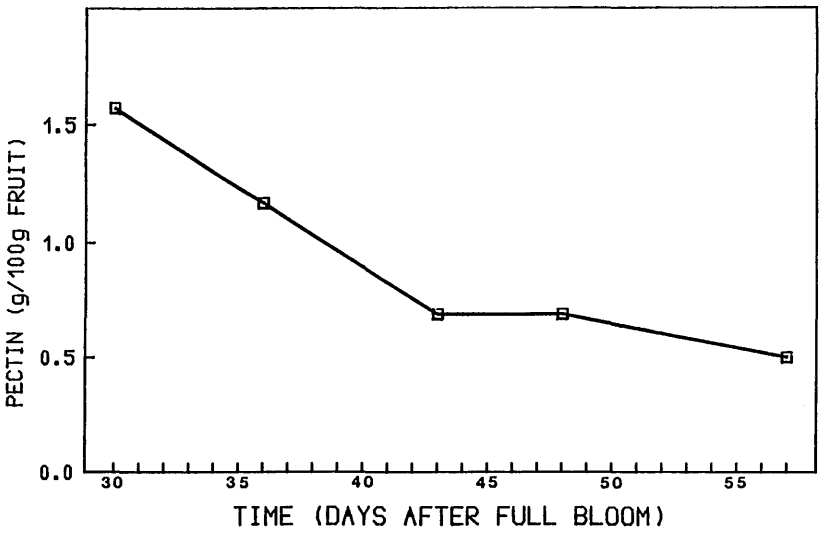


Figure 4. Changes in the pectin fractions of alcohol insoluble solids (AIS) derived from ripening "Bluetta" blueberries. WSP=Water soluble pectin (LSD=2.09), CSP=Chelator soluble pectin (LSD=1.74) and DASP=Dilute alkali soluble pectin (LSD=2.49).

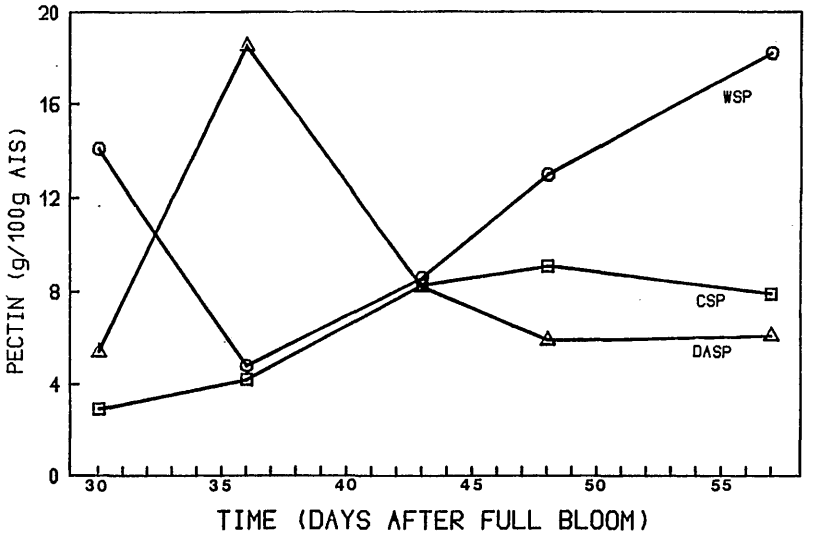
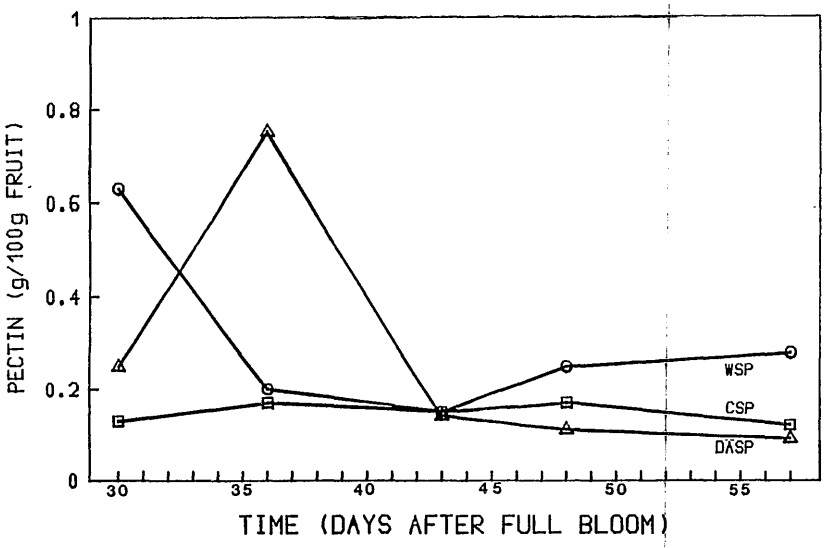


Figure 5. Changes in the pectin fractions of "Bluetta" blueberries expressed on a fruit fresh weight basis. WSP=Water soluble pectin (LSD=0.11), CSP=Chelator soluble pectin (LSD=0.04) and DASP=Dilute alkali soluble pectin (LSD=0.11).



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Changes in β-Carotene and Lipid Composition of Sweet Potatoes During Storage

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Introduction

Although lipids are present at 1.5 to 2.5 percent (dry weight basis) in sweet potatoes (Walter and Purcell, 1974), their quality, quantity and composition are important to the sensory satisfaction, nutrition and keeping quality (Peng, 1974). Lipids of sweet potato and their changes upon storage have not been studied extensively. Alexandridis and Lopez (1979) and Walter Purcell (1974) reported the lipid changes of sweet potato flakes during processing and storage, Boggess *et al.* (1967, 1970) studied sweet potato lipids affected by controlled storage and varieties, and Tereshkovich and Newsom (1963) examined sweet potato tissue lipids. According to Boggess *et al.* (1967), changes in lipid and fatty acid composition of sweet potatoes may be closely related to changes in the quality during storage. β-carotene, the major pigment in sweet potato roots, is also very susceptible to oxidation because of its unsaturated chemical structure (Eskin, 1979).

This study was conducted to investigate the effect of lower oxygen storage on lipid and fatty acid composition and β-carotene of stored sweet potatoes.

Materials and Methods

Georgia Jet sweet potato (*Ipomoea batatas*) cuttings were planted at The Ohio State University Horticultural Research and Testing Area, Columbus, OH under standard cultural practices. Immediately after harvesting, the roots were cured for eight days at 30°C and 85 percent RH.

Two lots of sweet potatoes were used in this experiment. For the first lot, 15 roots approximately 500 g each were placed in each of three 10-L glass containers and stored at 15.5°C and 85 percent RH for the entire experiment. For the second lot, 15 roots were approximate weight of 500 g each were placed in each of the three gas-tight glass containers containing approximately 7 percent oxygen (Mattus and Hassan, 1968) in nitrogen for a 3-month storage period at 15.5°C and 85 percent RH. A continuous flow system of 85 mL/min (0.5 air exchange/1 hr) was maintained throughout the experiment.

Two roots were removed from each container at intervals of 0, 1, 2 and 3 months for analysis. Each root was peeled immediately after removal from the container and longitudinally cut into strips approximately 1 cm wide and 1 cm thick. All sample strips from the same lot (six roots) were thoroughly mixed, weighed in an equal amount of 20 g and 200 g into a plastic bag, and frozen until analysis (within a month).

Moisture was determined by weight difference after drying in a Precision-Thalco recirculating oven at 100±2°C for 18-20 hr.

Analysis of β-carotene

Determination of β-carotene: β-carotene was determined according to the method of Karawya *et al.* (1975). Twenty gram frozen sample (four replications) of sweet potato was extracted with methanol:petroleum ether (1:9, v/v) in a stoppered flask for 15 min with occasional shaking. The extraction was repeated three times. The combined extracts were concentrated by a rotary evaporator at reduced pressure at 30°C, and the volume was adjusted to 10 mL with the same solvent in a volumetric flask.

TLC Spectrophotometric Assay of β-carotene: One tenth milliliter extract was chromatographed to a height of 10 cm on a glass plate (10 X 20 cm) coated with silica Gel G (Brinkmann Instruments, Westbury, NY) at a thickness of 250 μ in a petroleum ether-benzene (98:2, v/v) solvent system. The yellowish β-carotene spot was removed and quantitatively transferred to a 50 mL Erlenmeyer flask. After adding 20 mL of benzene, the mixture was stirred with a magnetic stirrer for 10 min, filtered through Whatman No. 1 paper into a 25 mL volumetric flask, and brought to volume with benzene. Absorbance of the solution was measured by a Beckman DU-2 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) at 464 nm against a blank using the

sample-free adsorbent material at the same height and size as the β-carotene spots on TLC plate.

Lipid Analysis

Extraction of Lipids: Four replicates of a 200 g frozen sample were extracted twice with Folch reagent (Folch *et al.*, 1957), consisting of chloroform-methanol (2:1, v/v) according to Peng (1974). The combined extract was quantitatively transferred to a separatory funnel and allowed to stand until two phases separated. The lower chloroform phase was collected and the upper alcohol phase was extracted with 30 mL chloroform and combined with the lower phase. The solvent was removed by a rotary evaporator at reduced pressure at 30°C and the sample stored in a vacuum desiccator until a constant weight was obtained.

Column Chromatography: The crude lipid extracts were separated into three classes (neutral lipids, glycolipids and phospholipids) by column chromatography. Silicic acid (Rouser *et al.*, 1967) was used at a sample loading ratio of 2:100 g adsorbent in an 11 mm diameter glass column with a 250 mL reservoir flask. Neutral lipids were eluted by chloroform at an elution ratio of 25 mL solvent/g adsorbent with a flow rate of 0.5 mL/min. The glycolipids were eluted by acetone at 40 mL/g adsorbent and phospholipids eluted by methanol at 25 mL/g adsorbent with the same flow rate.

Thin Layer Chromatograph (TLC): TLC was used to monitor the purity of each lipid class. Glass plates (10 X 20 cm) were coated with silica Gel G (Brinkmann Instruments, Westbury, NY) at a thickness of 250 μ. The developing solvent system for neutral lipids was chloroform and the plates were sprayed with phosphomolybdic acid. Chloroform-acetone-methanol-acetic acid-water (65:20:10:10:3, v/v) (Lepage, 1968) was used for polar lipids. An α-naphthol reagent (Siakotos and Rouser, 1965) was used for detecting glycolipids, and ninhydrin (Mangold, 1961) was used to identify phospholipids.

Gas-Liquid Chromatography (GLC): Fatty acid composition was analyzed by a Packard model 409 Becker gas chromatograph (Packard Instrument Company, Downers Grove, IL) equipped with a flame ionization detector, a Varian Aerograph series recorder (Varian Aerograph, Walnut Creek, CA) and an Autolab 6300 digital integrator (Autolab, Mountain View, CA). Methyl esters of fatty acids were prepared according to Metcalf *et al.* (1966) using borontrifluoride-methanol and were separated on a stainless steel column (244 cm X 0.3 cm OD) packed by Applied Science, Deerfield, IL, with 15 percent w/w Hi-EFF-1BP and 1 percent w/w phosphoric acid on Chromosorb W-AW, 80/100 mesh. The operating conditions were: column temperature, 190°C; FID detector temperature, 210°C; injection port temperature, 260°C; carrier gas, nitrogen at flow rate 26 mL/min. Methyl esters of fatty acids were identified by comparing retention time with authentic standards (Applied science, Deerfield, IL) and by plotting retention time vs. carbon number on semilog paper. The fatty acids were expressed as area percentage of the total area under the peaks of all methyl esters.

Sensory Evaluation

After each storage period, sweet potato samples were boiled for 1 hr and cooled to room temperature. Sensory quality including flavor, color and texture was evaluated by 10 untrained panelists on a 9-point hedonic scale (9=excellent, 1= poor). The data were statistically analyzed.

Statistical Analysis

Analysis of variance was performed. Comparison of means was accomplished by Tukey's method (1953) by the Statistical Analysis System (SAS, 1979).

Results and Discussion

β-carotene and Moisture: β-carotene of Georgia Jet sweet potatoes (2.4 mg/100 g) increased upon storage for one month at 15.5°C and 85 percent RH and then decreased. These results suggested that β-carotene was probably synthesized to a maximum during the first month, then declined thereafter due to oxidation. The decrease agreed with Miller and Covington (1942) who indicated that the -carotene of Porto Pico sweet potatoes increased rapidly during the first month of storage and remained constant after the second month. However, Picha (1985) found that total carotenoid content of four sweet potato cultivars (Centennial, Travis, Jasper, and Jewel) increased up to 16 weeks of storage at 15.6°C, and the initial concentration of carotenoids was also higher (5.0 to 9.5 mg/100 g). This discrepancy could be due to the differences in cultivars and curing conditions. There was no statistically significant difference between the means of the β-carotene of samples stored under atmospheric condition and those stored under low oxygen.

Moisture of the sweet potato samples was not influenced by treatment. Mean moisture of the two samples stored for 3 months at 15.5°C was 81.3 and 81.2 percent for atmospheric condition and low oxygen, respectively.

Total Lipids and Lipid Classes: Total lipids and their classes of Georgia Jet sweet potatoes are given in Table 1. Total lipids of fresh samples were 0.6 percent on a dry weight basis, lower than previously reported in Centennial sweet potatoes (Walter *et al.*, 1971; Boggess *et al.*, 1967, 1970; Alexandridis and Lopez, 1979). Total lipids from both treatments significantly increased after the first month of storage, probably due to lipid compositional changes during storage or synthesis from non-lipid components (Boggess *et al.*, 1967). The amount of neutral lipids was 38.6 percent, followed by glycolipids, 38.2 percent; and phospholipids, 23.2 percent. Neutral lipids of stored roots were markedly increased under two storage conditions at the expense of polar lipids, glycolipids and phospholipids. Although treatment did not significantly affect the levels of total lipids and lipid fractions, storage time affected their distributions.

Fatty Acid Composition of Fresh Sweet Potatoes: Fatty acid composition of total lipids and individual lipid classes of fresh Georgia Jet sweet potatoes primarily contained palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids, which constituted 91.7, 96.8, 89.5 and 95.9 percent of total fatty acids in total lipids, neutral lipids, glycolipids and phospholipids, respectively (Tables 2, 3). Linoleic was the predominant acid, followed by palmitic acid. These fatty acids are typical in plant lipids (Hitchcock and Nichols, 1971).

The lipids of Georgia Jet sweet potatoes were high in unsaturated fatty acid, which accounted for 66.5 percent of total fatty acids in total lipids, 64.8 percent in neutral lipids, 64.7 percent in glycolipids and 60.1 percent in phospholipids, respectively (Tables 2, 3). The primary saturated fatty acids were palmitic and stearic acids, which made up 26.8 and 26.1 percent of the total lipids and glycolipids, and over 30 percent of the neutral lipids and phospholipids. The fatty acid distribution pattern of total lipids and neutral lipids was similar. This result would be expected since neutral lipids were the major lipid fraction.

Changes in Fatty Acid Composition During Storage: Changes in fatty acid composition of total lipids and each lipid class are shown in Tables 2 and 3. Linoleic acid (18:2) decreased markedly from the original concentration in all samples of both treatments, but increased at the end of the storage period, especially in samples stored under low oxygen. Of the two treated samples, linoleic acid (18:2) in phospholipids decreased after the first month of storage and then increased throughout the rest of the storage period (Table 3).

Regardless of storage conditions, palmitic acid (16:0) in total lipids, neutral lipids and phospholipids decreased rapidly during the first month, then increased for the rest of the storage period (Tables 2, 3). The concentration of palmitic acid in the glycolipids in samples stored under atmospheric condition decreased slightly during the first month and remained constant for the remaining storage period. The concentration of this fatty acid increased slightly when stored under low oxygen condition (Table 3). The increase in the concentration of palmitic acid (16:0) may be due to synthesis from short chain fatty acids.

Stearic acid (18:0) increased progressively from the original concentration during storage in total and three lipid classes for both storage treatments. This increase reached the peak at the two month storage under atmosphere while the low oxygen treatment maintained the increase throughout the storage period (Tables 2, 3).

Concentration for linolenic acid (18:3) for both storage treatments varied with storage time in total and neutral lipids (Tables 2, 3), and increased significantly in glycolipids and phospholipids during the first month, then declined at the end of the storage period. Since this fatty acid is highly unsaturated and can readily undergo oxidation, the decrease would be anticipated. The results in this study indicated that synthesis of other fatty acids might have occurred in the stored roots. This is supported by Tereshkovich and Newsom (1963) who reported changes in fatty acid composition in periderm tissue of stored sweet potatoes.

The analysis of variance for selected fatty acids of sweet potato samples revealed that palmitic (16:0), stearic (18:0) and oleic (18:1) acids were affected significantly ($P < 0.01$) by the treatment. Both storage time and lipid classes significantly affected most fatty acids.

Changes in the Unsaturation Ratio of Sweet Potato Samples: The unsaturation ratio, which is expressed as the ratio of linoleic (18:2) plus linolenic (18:3) acids to palmitic

Table 1. Changes in total lipids and lipid classes of sweet potato during storage at 15.5°C and 85% RH

Storage Time mo	Total lipids[1,2] % dry weight		Lipid Class (% of total lipids)[1,2]					
			Neutral lipids		Glycolipids		Phospholipids	
	Atmospheric	Low oxygen	Atmospheric	Low oxygen	Atmospheric	Low oxygen	Atmospheric	Low oxygen
0	0.6[a]	0.6[a]	38.6[a]	38.6[a]	38.2[b]	38.2[b]	23.2[b]	23.2[b]
1	1.1[c]	1.0[bc]	51.0[b]	49.9[ab]	33.8[ab]	35.0[ab]	15.2[a]	15.1[a]
2	1.1[c]	1.1[c]	52.0[b]	53.4[b]	33.8[ab]	35.8[ab]	14.2[a]	10.8[a]
3	0.8[ab]	1.0[bc]	60.0[c]	55.4[b]	28.0[a]	32.2[a]	12.0[a]	12.4[a]
Mean[5]	0.9n.s.	0.9n.s.	50.4n.s.	49.3n.s.	33.5n.s.	35.3n.s.	16.0 n.s.	15.4n.s.

[1]Mean of 4 determinations
[2]Any means within the same column followed the same superscript are not significantly different ($P<0.05$), n.s.=not significant

Table 2. Changes in fatty acid composition of total lipids of sweet potato during storage at 15.5°C and 85% RH

Fatty acid[1]	Storage Condition						
	Fresh	Atmospheric			Low oxygen		
	0 mo	1 mo	2 mo	3 mo	1 mo	2 mo	3 mo
		(% total fatty acids)[2]			(% total fatty acids)[2]		
12.0	1.3	3.2	1.8	2.9	2.2	4.0	2.2
16:0	22.6	22.0	23.7	25.2	22.5	25.8	29.1
16:1	—	1.4	1.6	1.3	1.1	1.7	—
17:0	1.0	tr	1.6	2.4	0.7	2.9	tr
18:0	4.2	9.9	10.2	8.5	9.1	10.5	13.0
18:1	2.1	1.2	2.8	2.7	3.8	3.1	—
18:2	55.3	44.1	40.4	41.5	39.7	34.1	45.5
20.0	tr	1.2	2.5	3.4	4.5	4.3	tr
18:3	7.5	9.7	8.3	7.1	8.4	7.3	7.4
22:0	3.6	tr	1.5	—	0.6	—	tr
24:0	tr	3.0	tr	tr	—	—	—

[1]Carbon number:number of double bonds.
[2]Mean of 4 determinations.

(16:0) plus stearic (18:0) acids, had been used as an index of autoxidation of fatty acids of sweet potato flakes by Walter and Purcell (1974) and Alexandridis and Lopez (1979). Linoleic (18:2) and linolenic (18:3) acids represented almost 95 percent of the unsaturated fatty acids in total lipids. The oxidation rate of these two unsaturated fatty acids in potato granules was very rapid as previously reported (Buttery *et al.*, 1961). Therefore, the unsaturation ratio as defined above would be an appropriate index for the measurement of oxidation occurring in the stored sweet potato samples.

The unsaturation ratio of different lipid classes of Georgia Jet sweet potatoes is given in Table 4. This ratio decreased significantly upon storage. Storage conditions also significantly affected ($P<0.05$) the unsaturation ratio. A higher unsaturation ratio was found in samples stored under atmospheric condition. The increase in concentration of palmitic (16:0) and stearic (18:0) acids in samples stored under low oxygen condition (Tables 2, 3) resulted in a lower unsaturation ratio.

Sensory Evaluation of Sweet Potato Samples: Due to high variation in rating for flavor, color and texture of sweet potato samples, there were no statistically significant differences between samples stored under atmospheric and low oxygen conditions for three months. Although not significantly different, mean scores of samples under low oxygen condition were slightly higher than those of samples under atmospheric condition in flavor and color evaluation, 6.2-6.3 to 5.6. The results obtained from sensory evaluation of sweet potato samples in this study are supported by the work of Hoover *et al.* (1983), who indicated that sweet potato patties of acceptable quality could be prepared from either

harvested sweet potatoes or from roots that had been cured and stored for up to six months. Since there was no significant difference in color of sample upon storage for one, two, and three months, it could be speculated that the color of both samples was not influenced by the storage.

The β-carotene content of Georgia Jet sweet potatoes increased during storage. Neutral lipids were increased at the expense of glycolipids and phospholipids. Changes in fatty acid composition and distribution were found during storage, the unsaturation ratio declined due to an increase in palmitic and stearic acids. Since lipids are flavor precursors, changes in fatty acids are possibly related to the sensory properties of stored roots (Alexandridis and Lopez, 1979; Boggess *et al.*, 1967).

Table 3: Changes in fatty acid composition of sweet potato during storage at 15.5°C and 85% RH

	Storage Condition						
		Atmospheric			Low oxygen		
Fatty Acid[1]	Fresh 0 mo	1 mo (% of total fatty acids)[2]	2 mo	3 mo	1 mo (% of total fatty acids)[2]	2 mo	3 mo
Neutral Lipids							
16:0	25.7	18.9	26.3	27.2	21.7	29.7	28.1
16:1	—	tr	1.2	1.0	1.2	1.9	tr
18:0	7.9	11.7	11.7	9.8	9.9	11.2	15.2
18:1	1.4	4.6	3.8	3.2	—	3.4	—
18:2	53.6	34.4	37.6	42.0	42.9	35.7	46.9
20:0	tr	2.3	4.3	3.7	4.4	3.9	tr
18:3	8.2	5.8	8.5	7.4	9.2	6.9	6.5
22:0	0.8	8.7	tr	0.7	3.8	—	—
Glycolipids							
10:0	2.3	2.1	tr	tr	2.9	3.9	0.6
12:0	3.5	3.4	7.9	7.9	2.7	9.6	7.6
13:0	—	1.7	2.7	—	0.8	—	—
14:0	tr	1.0	tr	2.2	5.0	3.0	2.0
15:0	0.6	4.3	2.6	2.3	1.8	1.8	—
16:0	18.8	15.4	15.0	15.6	14.9	16.4	18.8
16:1	—	1.8	0.6	1.0	3.4	3.2	1.8
17:0	2.1	3.6	5.8	9.3	2.4	7.5	6.9
18:0	7.3	8.3	9.4	8.6	9.4	6.3	13.1
18:1	0.9	1.3	5.2	3.1	3.6	1.6	—
18:2	54.0	37.6	34.3	34.3	37.5	33.8	43.9
18:3	8.5	11.4	9.4	6.8	9.1	8.8	5.0
Phospholipids							
10:0	tr	2.3	1.1	tr	—	—	—
12:0	tr	2.2	2.0	2.2	1.1	0.9	—
15:0	tr	2.0	1.2	1.3	1.9	1.3	1.1
16:0	32.0	19.2	22.9	25.7	24.5	25.0	28.9
16:1	—	4.3	2.4	2.4	1.0	3.1	—
17:0	0.8	—	0.8	—	tr	—	2.1
18:0	5.7	9.5	8.4	9.3	9.6	9.4	16.4
18:1	2.1	4.7	4.1	5.5	4.6	3.6	tr
18:2	54.4	36.0	40.5	42.4	40.1	42.5	44.7
20:0	tr	6.9	5.0	2.5	5.4	2.5	—
18:3	1.6	6.5	7.1	6.6	7.5	6.7	6.0

[1]Carbon number:number of double bonds.
[2]Mean of 4 determinations.

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Table 4. Unsaturation ratio[1] of sweet potatoes during storage at 15.5°C and 85% RH

Storage	Atmospheric condition					Low oxygen				
Time mo	Total lipids	Neutral lipids	Glyco-lipids	Phospho-lipids	Mean[2,3]	Total lipids	Neutral lipids	Glyco-lipids	Phospho-lipids	Mean[2,3]
0	2.4	1.9	2.4	1.5	2.1[b]	2.4	1.9	2.4	1.5	2.1[b]
1	1.7	1.5	2.1	1.9	1.8[ab]	1.6	1.7	1.8	1.4	1.6[ab]
2	1.4	1.2	1.8	1.5	1.5[a]	1.2	1.1	1.6	1.4	1.3[a]
3	1.4	1.4	1.7	1.4	1.5[a]	1.3	1.2	1.6	1.4	1.4[a]
Mean	1.7	1.5	2.0	1.6	1.7[4]	1.6	1.5	1.9	1.5	1.6[4]

[1]Expressed as a ratio of linoleic +linolenic/palmitic+stearic.
[2]Mean of 4 determinations.
[3]Any means followed by the same superscript are not significantly different ($P<0.05$) between two storage conditions.
[4]Significantly different ($P<0.05$).

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Greenhouse Tomato Lipids

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Introduction

Americans are changing their eating habits and consuming more fresh fruits and vegetables. The tomato is one of the popular vegetables in the supermarket today. The peculiarity of tomato results from it being consumed either as a fruit or as a vegetable and the diversity of its products. It is served fresh, cooked, or processed such as canned, beverage, paste, and catchup. Generally, a ripe tomato contains 93.5 percent water, 1.1 percent proteins, 0.2 percent lipids, and 4.7 percent carbohydrates (Watt and Merrill, 1963), however, the composition of different cultivars may vary. Lipids, even though present in very low concentrations, are important to organoleptic satisfaction and keeping quality (Litman and Numrych, 1978).

Most studies on tomato lipids have been with field-grown tomatoes. Tomato leaves are high in glycolipids and low in phospholipids (Roughan and Batt, 1969), and linoleic, oleic, and palmitic acids are the predominant fatty acids in tomato seeds (Miric and Cupic, 1966). Changes in neutral lipids, glycolipids, and phospholipids during maturation have been reported (Ueda *et al.*, 1970; Minamide *et al.*, 1970; Minamide and Ogata, 1972a, 1972b). Minamide *et al.* (1970) also studied the phospholipids and their fatty acid composition of a glass house tomato.

This study was undertaken to investigate neutral lipids, glycolipids, and phospholipids and their fatty acid composition of tomatoes grown in the greenhouse.

Materials and Methods

Tomato (*Lycopersicon esculentum*) cultivars, GS244, MR13, and Jumbo were grown in The Ohio State University horticultural greenhouse with standard cultural practices. Tomatoes were harvested when they were red-ripe.

One kilogram of tomatoes from each cultivar were cleaned, core and seeds were removed. The fruits were cut into approximately 2 x 2 cm and mixed thoroughly for even distribution of lipids. Samples (200 g) were placed in a plastic bag and frozen until analysis (within a month). The moisture content was determined by weight difference before and after heating in a Precision-Thelco recirculating oven at 100±2°C for 15 hr.

Duplicate frozen samples were extracted with Folch reagent (Folch *et al.*, 1957) as previously reported (Peng, 1982). Total lipids were separated into three classes by silicic acid column chromatography and monitored by thin-layer chromatography (Peng, 1974, 1982). Methylesters of fatty acids of each lipid fraction were prepared by boron-trifluoride methanol (Metcalfe *et al.*, 1966). Qualitative and quantitative analyses were performed by Packard model 409 Becker gas chromatograph (Packard Instrument, Downers Grove, IL) equipped with a flame ionization detector and HP 3390A reporting integrator (Peng, 1974, 1982). Identification of fatty acid methylesters was made by comparing retention time of reference methylesters, and by plotting retention time vs. carbon number on semilog paper for those other than references analyzed on the same column under the same conditions. The peak areas corresponding to the fatty acids were expressed as a percentage of the total peak area from all methylesters.

Results and Discussion

The moisture content of three tomato cultivars ranged from 94.0 to 95.2 percent which was higher than reported for the field-grown tomatoes, but agreed with the glass house tomatoes (Ueda *et al.*, 1970).

Total lipids and lipid components illustrated a varietal effect on composition and distribution as shown in Table 1. A higher concentration of glycolipids appears to be characteristic of typical photosynthetic plants (Hitchcock and Nichols, 1971). GS244 was high in neutral lipids and low in glycolipids. The total lipid content was very low, only 0.07 to 0.11 percent.

The fatty acid composition (Table 2) showed a typical plant lipid pattern (Hitchcock and Nichols, 1971) in which palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) were the major fatty acids, while erucic (22:1) was the minor one. Cultivars MR13 and

Jumbo had higher concentrations of all five major acids (49.35 percent and 55.60 percent for neutral lipids, 73.70 percent and 70.25 percent for glycolipids, and 75.35 percent and 77.25 percent for phospholipids) than GS244 (43.30 percent, 61.85 percent, and 71.05 percent, respectively). Phospholipids contained the highest concentration of the five fatty acids and neutral lipids the lowest, in all three cultivars.

There seems to be a definite distribution pattern of the five acids (Table 2). In neutral lipids, except GS244, the pattern was 18:2>16:0>18:1>18:3>18:0 (the ratio was 0.32:0.25:0.17:0.13:0.13 for MR13 and 0.28: 0.26:0.17:0.16:0.13 for Jumbo), with linoleic acid being the leading fatty acid. In glycolipids, linolenic acid was the highest, with the profile being 18:3>18:2 >16:0>18:1>18:0. The phospholipids had a distribution pattern, 18:2>18:3>16:0>18:0>18:1 which agrees with other findings (Minamide *et al.*, 1970). However, none of the greenhouse tomato fatty acid distribution patterns complied with field tomatoes (Ueta *et al.*, 1970; Minamide *et al.*, 1970; Minamide and Ogata, 1972). Environmental factors may play a significant role (Hitchcock and Nichols, 1971).

Table 1. Lipid content of greenhouse tomatoes[1]

	Neutral lipids		Glycolipids		Phospholipids	
Cultivar	Wt(mg)	%	Wt(mg)	%	Wt(mg)	%
GS244	56.4	48.66	29.9	25.79	29.6	25.55
MR13	27.4	29.82	39.4	42.87	25.1	27.31
Jumbo	14.8	28.13	35.4	40.41	27.4	31.46

[1]Average of two determinations.

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Table 2. Fatty acid composition of greenhouse tomatoes

	Fatty acid (%)[1]							
Cultivar	12:0	14:0	16:0	18:0	18:1	18:2	18:3	22:1
Neutral lipids								
GS244	1.65±0.15	3.65±0.15	17.85±1.65	4.15±0.75	5.55±1.75	13.30±2.00	2.45±0.15	2.85±1.25
MR13	2.15±0.25	4.50±0.20	12.30±0.40	6.45±0.05	8.55±0.15	15.55±0.25	6.50±0.10	1.15±0.15
Jumbo	2.45±0.45	4.45±0.35	14.45±1.65	7.10±1.10	9.30±0.00	15.80±1.10	8.95±1.15	1.25±0.05
Glycolipids								
GS244	5.05±0.65	1.55±0.15	14.40±0.70	4.20±0.50	4.10±0.10	15.25±0.05	23.90±0.90	—
MR13	2.90±0.10	1.75±0.05	14.20±0.00	8.20±0.50	8.20±0.30	19.30±1.00	23.80±0.20	1.00±0.20
Jumbo	1.05±0.15	0.60±0.00	14.70±1.50	4.35±0.25	7.00±1.10	20.35±0.55	23.85±1.05	2.60±0.00
Phospholipids								
GS244	2.20±0.00	1.35±0.05	15.70±0.40	3.80±0.10	2.70±0.00	27.25±0.65	21.60±0.30	—
MR13	0.90±0.00	0.30±0.00	15.25±0.75	8.25±0.15	5.20±0.40	25.00±0.40	21.65±0.45	1.30±0.10
Jumbo	0.75±0.15	0.30±0.10	18.15±0.05	4.65±0.35	2.90±0.00	29.45±0.05	22.10±0.40	1.65±0.15

[1]Carbon number:number of double bonds

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Highbush Blueberry Fatty Acids and Lipid Unsaturation Ratio

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Introduction

Commercial highbush berries are a substantial and fast-growing fruit crop industry in the United States (Eck, 1988). However, limited literature is available defining blueberry quality factors in relation to the chemical composition of the fruit. Studies have shown that berry quality is closely associated to genetic background and the changes in chemical characteristics of blueberries (Woodruff *et al.*, 1960; Sapers *et al.*, 1984).

Lipids are the main component of cell membrane and are affected by environmental stress. Changes in the composition of membrane lipid classes in response to temperature, oxygen, chemical and radiation stresses have been reported (Hitchcock and Nichols, 1971; Geduspan and Peng, 1987; Ramarathnam *et al.*, 1989). Studies of plant freezing injury and resistance indicate that the primary site of damage is the cell membrane (Palta and Li, 1978) and that the membrane undergo changes in the composition of phospholipids. Cultivars may differ with respect to cold hardiness. The relative cold hardiness of different cultivars has been assessed (Eck, 1988). The aim of this research was to study the fatty acids and their unsaturation ratio of five highbush blueberries which may be useful in understanding of membrane lipid composition in relation to cold hardiness.

Materials and Methods

Materials: Five commercial blueberry cultivars; three late maturing cultivars, Coville, Darrow, Herbert; and two early maturing cultivars, Jersey and Bluetta were harvested and frozen at −10°C.

Lipid Extraction: Frozen samples were rinsed with tap water to remove detached pedicels. Lipid was extracted by an adaption of the method of Bligh and Dyer (1959). Duplicate 100 gram samples were blended directly with 300 ml of Folch reagent (Folch *et al.*, 1957) consisting of chloroform and methanol (2:1, v/v) for 4 minutes at room temperature. Chloroform extracts were then washed twice with 0.9 percent salt solution. The extract was concentrated by rotary evaporation under reduced pressure. The temperature was maintained at less than 30°C.

Separation of Lipid Class

Silicic Acid Column Chromatography: Crude lipids were separated into three classes by silicic acid column (Rouser *et al.*, 1967). The non-polar lipids were isolated from polar lipids by a sample loading ratio of 2:100 g silicic acid (adsorbent) in a 1.1 cm diameter glass column. A 100 mg sample of crude lipid extract was dissolved in 5 ml of chloroform and applied to the column. Eluting solvents, 80 ml chloroform, 60 ml acetone, and 60 ml methanol were applied in that order respectively. The flow rate throughout was 0.5 ml per minute. Solvent was removed with the aid of an evaporator at a reduced pressure.

Thin Layer Chromatography: The purity of each fraction was monitored by thin layer chromatography. Non-polar lipids were developed with chloroform and sprayed with phosphomolybdic acid. Polar lipids were developed by Lepage solvent system (Lepage, 1967). Ninhydrin reagent was used for detecting phospholipids and 0.5 percent α-naphthol for detecting glycolipids.

Gas Liquid Chromatography: Methyl ester derivatives of the fatty acids of neutral lipid, glycolipid and phospholipid fractions were prepared according to Metcalfe *et al.* (1966). Fatty acid composition was determined using a gas liquid chromatograph (Hewlett Packard model 5890A) with a flame ionization detector (FID) and a Hewlett Packard model 3390A integrator. The stainless steel column (305 × 0.32 cm OD) was prepacked with a 15 percent (w/w), diethyleneglycol succinate and 1 percent (w/w), phosphoric acid in a solid support phase of acid-washed Chromosorb W, 80-100 mesh. The column temperature was programmed at the rate of 10°C per minute from 80°C to increase to a maximum 190°C. Detector temperature was

230°C and the injection port temperature was 200°C. The carrier gas was nitrogen and its flow rate was 20 ml per minute.

Fatty acid composition of each lipid fraction on the chromatogram was identified by comparing the retention time of reference compounds, and plotting retention time vs. carbon number on semilog paper for those other than reference compounds analyzed on the same column under the same operating conditions.

Statistical Analysis: Significance between unsaturated/saturated ratios of various lipid classes was analyzed by Analysis of Variance (ANOVA). Simple linear correlation coefficients, r, were computed for the changes in the unsaturated/saturated ratio of each lipid class as a function of maturing date.

Results and Discussion

The fatty acid composition of five cultivars revealed that myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) were the principal fatty acids (Tables 1-5). In particular, polyunsaturated acids, linoleic (40-45 percent), oleic (18-23 percent) and linolenic acids (17-24 percent) account for 82-87 percent of all fatty acids. Some minor fatty acids were capric (10:0), lauric (12:0), palmitoleic (16:1), heneicosanoic (21:0), behenic (22:0) and lignoceric (24:0), present in concentration less than 1 percent of total lipids. The patterns of major fatty acid composition of five cultivars were fairly similar with respect to their total lipids and individual lipid class.

Table 1. Fatty acid composition of Coville blueberry (area percentage).

Fatty Acids	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
10:0[1]	0.23	0.47	—	0.21
11:0	0.04	0.54	—	—
12:0	0.14	0.22	0.39	0.76
14:0	1.22	2.04	0.98	2.01
15:0	—	0.23	0.41	0.71
16:0	12.95	7.02	18.86	21.24
16.1	—	0.06	0.12	0.15
16:3	—	—	—	0.36
18:0	2.35	2.67	3.39	2.77
18:1	17.60	22.96	7.44	8.01
18:2	44.44	36.87	47.66	49.60
18:3	19.62	23.85	20.06	14.04
21:0	0.26	1.78	0.32	—
22:0	0.33	0.65	0.25	0.14
23:0	0.48	0.21	—	—
24:0	0.32	0.38	—	—
U/S[2]	4.45	5.15	3.05	2.59

[1]Carbon number: number of double bond.
[2]Unsaturated/saturated fatty acids

Table 2. Fatty acid composition of Darrow blueberry (area percentage).

Fatty Acids	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
10:0[1]	0.08	0.42	—	—
11:0	—	0.08	—	—
12.0	0.15	0.11	0.97	—
14.0	2.27	1.22	1.94	0.47
15.0	0.28	—	0.15	—
16:0	12.69	7.33	21.82	21.23
16:1	—	—	0.17	0.20
16:3	0.04	—	—	0.22
18:0	2.31	2.09	4.42	3.09
18:1	18.87	24.33	9.50	11.41
18:2	40.12	37.73	28.74	48.88
18:3	22.48	24.84	31.62	14.19
21:0	0.20	0.33	0.45	—
22:0	0.25	0.23	0.22	0.30
23:0	—	0.51	—	—
24:0	0.22	0.53	—	—
U/S[2]	4.41	6.69	2.34	2.95

[1]Carbon number: number of double bond.
[2]Unsaturated/saturated fatty acids.

Table 3. Fatty acid composition of Herbert blueberry (area percentage).

Fatty Acids	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
10:0[1]	0.11	0.25	—	—
11:0	—	0.15	—	0.23
12:0	0.12	0.20	0.88	0.56
14:0	2.31	1.75	1.64	3.75
15:0	0.38	0.09	0.15	—
16:0	12.13	9.24	18.31	21.76
16:1	—	—	0.18	0.12
18:0	3.46	2.37	4.56	3.68
18:1	22.51	23.17	8.59	10.22
18:2	40.60	37.43	43.66	43.70
18:3	16.56	24.14	21.43	14.64
21:0	0.28	0.70	0.27	0.15
22:0	1.06	0.17	0.27	0.15
23:0	0.21	0.15	—	—
24:0	0.25	—	—	—
U/S[2]	3.92	5.55	2.83	2.29

[1]Carbon number: number of double bond.
[2]Unsaturated/saturated fatty acids.

Table 4. Fatty acid composition of Jersey blueberry (area percentage).

Fatty Acids	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
10:0[1]	0.04	0.40	0.31	—
11:0	—	0.12	0.23	—
12:0	—	0.12	0.96	0.41
14:0	1.09	0.57	2.31	1.86
15:0	—	0.11	1.01	1.06
16:0	9.44	7.35	22.18	16.89
16:1	—	—	0.25	0.33
18:0	2.05	1.93	5.36	2.82
18:1	20.44	23.36	7.18	7.35
18:2	45.15	43.77	27.94	51.86
18:3	20.15	20.67	31.82	16.87
21:0	0.12	0.41	0.07	0.13
22:0	1.09	0.44	0.37	0.39
23:0	—	0.37	—	—
24:0	0.21	0.36	—	—
U/S[2]	6.01	7.20	2.05	3.24

[1]Carbon number: number of double bond.
[2]Unsaturated/saturated fatty acids.

Varietal Difference on the Unsaturation Ratios of Fatty Acids of Lipid Classes: Three types of ratio of unsaturated to saturated fatty acids, R1, R2, and R3, were used as indicators of varietal differences in total lipids, neutral lipids, glycolipids and phospholipids (Table 6). R1 is the ratio of total unsaturated to saturated fatty acids; R2, the ratio of 18:1+18:2+18:3/16:0; and R3, the ratio of 18:1+18:2+18:3/18:0 +16:0.

The unsaturation ratios indicated that blueberry fruit lipids contained higher unsaturated fatty acids than saturated fatty acids. The average of R1 of five cultivars for neutral lipids, glycolipids and phospholipids were 6.80, 2.59 and 2.94, respectively. Neutral lipids exhibited highest unsaturation, followed by phospholipids and glycolipids, the lowest.

For total lipids, it was observed that R1, R2 and R3 decreased significantly with increase in the season ranking value ($P<0.05$). Simple correlation coefficients for R1, R2 and R3 were 0.81, 0.99 and 0.78 respectively. The ratio of (18:1+18:2+18:3)/16:0, R2, was the most conspicuous indicator of varietal differences in the total lipids. The unsaturation order was as follows: Bluetta > Jersey > Herbert=Darrow > Coville.

A similar trend was observed in neutral lipids that earlier maturing cultivars, Bluetta and Jersey have a higher unsaturation ratio. The most conspicuous indicator of lipid changes occurring in the neutral lipids was, R1, the ratio of total unsaturated/saturated fatty acids. In contrast to the other lipid fractions, no definite trend could be drawn for early maturing fruit showing a higher unsaturation ratios in glycolipids. For phospholipids, it was observed that R1, R2 and R3 decreased

significantly with increase in the season rating ($P<0.05$). Simple correlation coefficients for R1, R2 and R3 were 0.83, 0.75 and 0.80 respectively. Unsaturation ratio of five cultivars in phospholipids were in the following order:

Bluetta > Jersey > Darrow=Coville > Herbert

Studies of freezing injury and resistance in plants indicated that the primary site of freezing injury is the cell membrane (Palta and Li, 1978) and that the membranes undergo changes in both the total amount of the composition of phospholipids during the hardening.

Hatano and Kabata (1982) reported palmitic, oleic, linoleic and linolenic acids greatly increased and lipid bodies appeared during the hardening. They suggested that change in fatty acid synthesis is essential for the development of frost hardiness.

Wase and Bishop (1978) reported an increase in total unsaturated acids and a large increase in the double bond index of phospholipids as the fruit ripened. Further evidence was demonstrated by Kimura et al. (1982) that the fruits' lipid composition may determine its susceptibility to chilling injury. They found that the mitochondrial lipid of unsaturated fatty acids were higher in a chilling resistant cultivar of apples than in a chilling sensitive cultivar, and postulated that the differences in unsaturated fatty acid content would cause the difference in resistance to chilling injury by lowering the phase transition temperature.

Cultivars may differ in cold hardiness within a species of blueberry. The relative cold hardiness of different cultivars has been assessed by various methods (Eck, 1988). The hardiness ranking depends on the method used. The results in this study, according to their unsaturation ratios in phospholipids of five blueberry cultivars show that the ranking order is in agreement with experimental evidence by Quamme *et al.* (1972) based on a determination of the lowest survival temperature. Hence, the increase in the relative amounts of the lower melting point unsaturated fatty acids, oleic, linoleic and linolenic acids, in blueberry phospholipids may explain the increase in cold hardiness of a cultivar with a higher unsaturation in blueberry lipids.

Table 5. Fatty acid composition of Bluetta blueberry (area percentage).

Fatty Acids	Total Lipids	Neutral Lipids	Glycolipids	Phospholipids
10.0[1]	0.10	0.21	0.29	—
11:0	—	0.06	—	—
12:0	0.12	0.13	0.39	0.27
14:0	1.22	0.54	2.24	0.51
15:0	—	0.14	1.20	—
16:0	9.08	6.67	18.05	16.99
16:1	—	—	0.29	0.42
16:3	—	—	—	0.09
18:0	1.73	1.58	3.29	2.18
18:1	21.76	25.42	8.10	8.95
18:2	41.57	41.04	32.64	52.84
18:3	23.36	23.51	31.64	16.18
21:0	0.27	0.20	1.27	0.44
22:0	0.36	0.20	0.60	1.06
24:0	0.47	0.12	—	—
U/S[2]	6.46	8.97	2.66	3.65

[1]Carbon number: number of double bond.
[2]Unsaturated/saturated fatty acids.

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Table 6. Unsaturation Ratios[1] of Fatty Acids of Lipid Fractions among Five Blueberry Cultivars.

Lipid Fraction	Correlation[3]		Cultivar[2]				
	Ratio	r	Coville	Darrow	Herbert	Jersey	Bluetta
Total lipids	R_1	0.81	4.45[a]	4.41[a]	3.92[a]	6.01[b]	6.46[b]
	R_2	0.99	6.31[a]	6.54[a]	6.55[a]	7.07[ab]	9.01[b]
	R_3	0.78	5.34[a]	5.53[a]	5.12[a]	7.45[b]	7.64[b]
Neutral lipids	R_1	0.93	5.15[a]	6.69[a]	5.55[a]	7.20[b]	8.97[b]
	R_2	0.63	11.92[a]	11.86[a]	9.17[b]	11.95[a]	13.49[c]
	R_3	0.79	8.64[a]	9.23[a]	7.28[b]	9.46[a]	10.87[ac]
Glycolipids	R_1	—	3.05[a]	2.34[b]	2.83[a]	2.05[b]	2.66[ab]
	R_2	—	3.99[a]	3.20[ab]	4.02[a]	3.02[b]	4.01[a]
	R_3	—	3.38[a]	2.66[a]	3.22[a]	2.43[ab]	3.39[a]
Phospholipids	R_1	0.83	2.59[a]	2.95[ab]	2.29[a]	3.24[b]	3.65[b]
	R_2	0.75	3.37[a]	3.06[a]	3.15[a]	4.50[b]	4.59[b]
	R_3	0.80	2.98[a]	3.06[a]	2.69[a]	3.86[b]	4.07[b]

[1]R_1 =total unsaturated/saturated fatty acids.
R_2 =I8:1+I8:2+I8:3/I6:0.
R_3 =I8:1+I8:2+I8:3/I8:0+I6:0.
[2]Means with the same letter in the same row are not significantly different ($P>0.05$).
[3]Simple linear correlation coefficients, r, were computed for the changes in the unsaturated/saturated ratio as a function to maturing date.

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